



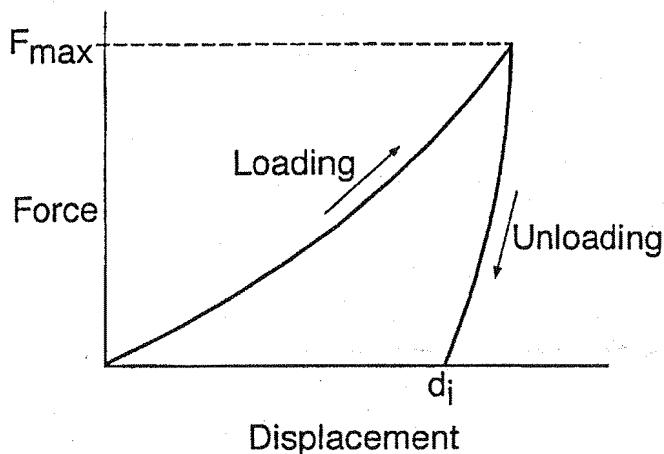
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A23G 9/02		A1	(11) International Publication Number: WO 00/53029
			(43) International Publication Date: 14 September 2000 (14.09.00)
(21) International Application Number: PCT/EP00/01760		(72) Inventors: DANIEL, Adrian; Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB). OLDROYD, Jon, Richard; Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB).	
(22) International Filing Date: 2 March 2000 (02.03.00)			
(30) Priority Data: 9905524.6 10 March 1999 (10.03.99) GB 99305842.9 23 July 1999 (23.07.99) EP		(74) Agent: EVANS, Jacqueline Gail Victoria; Unilever PLC, Patent Department, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB).	
(71) Applicant (for AE AU BB CA CY GB GD GH GM IE IL KE LC LK LS MN MW NZ SD SG SL SZ TT TZ UG ZA ZW only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except AE AU BB CA CY GB GD GH GM IE IL IN KE LC LK LS MN MW NZ SD SG SL SZ TT TZ UG ZA ZW): UNILEVER NV [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).			
(71) Applicant (for IN only): HINDUSTAN LEVER LIMITED [IN/IN]; Hindustan Lever House, 165/166 Backbay Reclamation, Mumbai 400 020, Maharashtra (IN).			
		Published With international search report.	

(54) Title: ICE CONFECTION CONTAINING ANTIFREEZE PROTEIN

(57) Abstract

An ice confection product having a volume of from 1 to 20 ml and a thickness of greater than 5mm comprising an antifreeze protein, wherein Δ modulus/original modulus ≥ 0.4 , and/or Δ strength/original strength ≥ 0.4 ; providing that when Δ modulus/original modulus ≤ 6.0 , Δ modulus ≥ 50 MPa, and/or when Δ strength/original strength ≤ 2.0 , Δ strength ≥ 0.2 MPa. Such ice confection products have properties which are akin to boiled sweets. Such products cannot be bitten or chewed but must be sucked, resulting in a product which remains in the mouth longer and is more refreshing.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

ICE CONFECTION CONTAINING ANTIFREEZE PROTEIN

Technical Field of the Invention

5 The invention relates to novel ice confections. In particular the invention relates to novel ice confections containing an antifreeze protein.

Background to the Invention

10 It is highly desirable to be able to manufacture ice confections having novel shapes, properties and/or textures. Until now, however the ability to provide such a high degree of novelty and interest to the products has been limited. In
15 particular products have to be manufactured with the ability to survive packaging, storage and distribution.

In the confectionery industry sweets such as boiled sweets and gobstoppers are very popular. However to date it has not
20 been possible to reproduce such products within a frozen confection.

The problem in preparing such frozen ice confection products is that when they are made of conventional frozen
25 confectionery mixtures, for example water ice mixtures or ice cream mixes, they do not have the desired eating characteristics. In particular such products tend to be rather wet at their surface, relatively soft to eat and not long lasting in the mouth. Also these products often cannot
30 be co-packed as they tend to stick together during storage.

We have now shown that inclusion of specific antifreeze proteins into selected ice confections results in the

- 2 -

formation of a strong, close-packed continuous network of ice crystals within the ice confection. As a result the ice confection is provided with specific defined mechanical properties. In particular the ice confection becomes hard
5 and once of a certain dimension becomes difficult to bite or chew, but must be sucked, exactly like a boiled sweet of the confectionery industry.

WO 98/04146 (Unilever) discloses that AFPs can be
10 incorporated into frozen food products such as ice confections to provide desirable product properties providing that the product and processing conditions are varied such that the ice crystals provided in the product have an aspect ratio of more than 1.9, preferably from 1.9
15 to 3.0. The specific examples given are all aerated ice cream compositions. As shown by comparative Examples A to C below, the addition of antifreeze proteins to aerated ice cream does not significantly change the mechanical properties of the ice cream. WO 98/04146 does not teach that
20 it is possible to provide specific ice confection products having novel mechanical properties and that such ice confections enable an ice confection equivalent to a boiled sweet to be manufactured.

25 WO 96/39878 (The Pillsbury Company) discloses a method for making a frozen composition for storage, the method not requiring a hardening step prior to storage. The frozen composition contains an antifreeze protein, in particular Type I AFP. Examples show the preparation of an aerated ice
30 cream and an aerated frozen yogurt. As shown by comparative Examples A to C below, the addition of antifreeze proteins

- 3 -

to aerated ice cream does not significantly change the mechanical properties of the ice cream. WO 96/39878 does not teach that it is possible to provide specific ice confection products having novel mechanical properties and that such ice confections enable an ice confection equivalent to a boiled sweet to be manufactured.

US 5 118 792 (Warren et al) discloses the addition of fusion proteins, and in particular the fusion protein protein A-Saf5 into foods which are to be consumed frozen, for example, ice cream, frozen yogurt, ice milk, sherbet, popsicles and frozen whipped cream. No examples are given where a final ice confection product is provided containing such fusion proteins. It is shown in Example 3B that when a popsicle formulation is used within the "splat assay", growth of the ice crystals is restricted.

Surprisingly we have now found that the addition of specific antifreeze proteins to defined ice confections, for example to water ices, ice milks, and unaerated ice cream, results in the formation of a strong, close-packed continuous network of ice crystals within the ice confection providing significant, advantageous changes to the mechanical properties of the ice confection. In particular an ice confection which is the frozen equivalent of the boiled sweet can be provided which has to be sucked and not bitten or chewed. As a result the product is retained for longer in the mouth and is found to be particularly refreshing.

- 4 -

Disclosure of the Invention

Accordingly the invention provides an ice confection product having a volume of from 1 to 20 ml and a thickness of
5 greater than 5mm comprising an antifreeze protein, wherein
 Δ modulus/original modulus ≥ 0.4 , and/or
 Δ strength/original strength ≥ 0.4 , providing that when
 Δ modulus/original modulus ≤ 6.0 , Δ modulus ≥ 50 MPa, and/or
when Δ strength/original strength ≤ 2.0 ,
10 Δ strength ≥ 0.2 MPa.

Preferably Δ modulus/original modulus ≥ 0.4 ; providing that
when Δ modulus/original modulus ≤ 6.0 , Δ modulus ≥ 90 MPa.

15 Most preferably Δ modulus/original modulus ≥ 1.0 ; providing
that when Δ modulus/original modulus ≤ 6.0 , Δ modulus \geq
100MPa.

Preferably Δ strength/original strength ≥ 0.7 , most
20 preferably Δ strength/original strength ≥ 1.5 .

By modulus is meant the apparent elastic modulus (E) as
determined using four point bend test. Example 1 gives the
standard procedure for performing a four point bend test.

25

Therefore Δ modulus (ΔE) means the change in modulus between
two ice confections whose formulation and process of
manufacture are identical in all respects except that the
first ice confection includes in its composition an
30 antifreeze protein, and the second ice confection has no

- 5 -

antifreeze protein included in its composition (the control composition). Original modulus (E_{orig}) is the modulus measured in the control composition.

5 By strength is meant the flexure strength (σ_u) which can be defined as the maximum stress that a material can withstand, under the particular conditions. The flexure strength is given by the stress at a point of maximum force on the force versus displacement curve recorded during a four point bend
10 test.

Therefore Δ strength ($\Delta\sigma_u$) means the change in strength between two ice confections whose formulation and process of manufacture are identical in all respects except that the
15 first ice confection includes in its composition an antifreeze protein, and the second ice confection has no antifreeze protein included in its composition (the control composition). Original strength ($\sigma_{u orig}$) is the modulus measured in the control composition.

20

In addition to changes in the apparent elastic modulus and flexure strength, an increase in product hardness is provided by the ice confections according to the invention.

25 For ice confections frozen with agitation, for example in an ice cream freezer (such as a scraped surface heat exchanger), the increase in hardness can be measured using the Vickers hardness test. Details of the Vickers hardness test are given in Example 3.

30

- 6 -

The degree to which the Vickers Hardness (H_v) of the ice confection is increased by the addition of the antifreeze protein depends in part on the ice content of the ice confection.

5

However, generally $\Delta H_v / H_{v \text{ orig}} \geq 0.3$, providing that when $\Delta H_v / H_{v \text{ orig}} \leq 5.0$, $\Delta H_v \geq 0.3$.

Preferably $\Delta H_v / H_{v \text{ orig}} \geq 1.0$, providing that when

10 $\Delta H_v / H_{v \text{ orig}} \leq 5.0$, $\Delta H_v \geq 1.25$.

Most preferably either $\Delta H_v / H_{v \text{ orig}} \geq 6.0$ or $\Delta H_v / H_{v \text{ orig}} \leq 6.0$ and $\Delta H_v \geq 2.0$.

15 Where ΔH_v is the change in Vickers Hardness between two ice confections whose formulation and process of manufacture are identical in all respects except that the first ice confection includes in its composition an antifreeze protein, and the second ice confection has no antifreeze
20 protein included in its composition (the control composition). $H_{v \text{ orig}}$ is the original Vickers Hardness measured in the control composition.

By close-packed continuous network of ice crystals is meant
25 that any given ice crystal is connected to at least one other ice crystal.

In unaerated ice confections which have been frozen with agitation, the degree of network formation can be measured
30 as contiguity. Contiguity is defined as the ratio of the

- 7 -

particle to particle interface area divided by the total interface area. It is thus a measure of the degree of network formation of the particle phase. Example 4 shows a method for the measurement of contiguity.

5

Un-aerated ice confections according to the invention have a contiguity of at least 0.2, as measured by the test given in Example 4, for an ice content when measured at -18°C of from 50-90%, preferably 54-85% by weight.

10

In un-aerated ice confections which have been frozen by any means, the degree of network formation can be measured as the Euler-Poincare characteristic of the ice phase. The Euler-Poincare characteristic is a measure of the degree of network formation of a particular phase. The lower and more negative the value of the Euler-Poincare characteristic, the greater the continuity of the phase in question. Example 5 shows a method for the measurement of the Euler-Poincare characteristic

15

Un-aerated ice confections according to the invention have an ice phase Euler-Poincare characteristic of less than -150 mm^{-2} , as measured by the test given in Example 5, for an ice content of from 50-90%, preferably 54-85% by weight, when measured at -18°C .

20

Products according to the invention are typically of comparable size and similar shapes to boiled sweets or ice cubes, such as rectangular chunks, cubes, spherical balls, small pyramids, ellipsoidal shapes, small animal shapes etc.

25

30

- 8 -

Preferably the thickness of the product is at least 1cm. Typically the thickness of the product will be in the range of from 1 to 4cm, more preferred from 1.5 to 3cm. Preferably the length of the product is at least 1cm. Typically the length of the product is from 1.5 to 6 cm, more preferred from 2.5 to 5cm.

Preferably the ice confectionery product has a volume of from 3 to 15ml, more preferred from 5 to 15ml.

The ice confectionery product of the invention may conveniently be sold in multi-packs like boiled sweets.

By antifreeze protein (AFP) is meant a protein which has significant ice recrystallisation inhibition properties as measured in accordance with Example 2. The AFP provides an ice particle size upon recrystallisation of less than 20 μ m, more preferred from 5 to 15 μ m.

Preferably the ice confection comprises at least 0.0005% by weight antifreeze protein, more preferably 0.0025% by weight antifreeze protein. Typically the ice confection will comprise from 0.0005% by weight to 0.005% by weight antifreeze protein.

For some applications it may be advantageous to include a mixture of two or more different AFPs into the food product.

The AFP for use in products of the invention can be any AFP suitable for use in food products. Examples of suitable sources of AFP are for example given in the article

- 9 -

"Antifreeze proteins and their potential use in frozen food products", Marilyn Griffith and K. Vanya Ewart, Biotechnology Advances, vol 13, pp375-402, 1995 and in patent applications WO 98/04699, WO 98/04146, WO 98/04147, 5 WO 98/04148 and WO 98/22591.

The AFPs can be obtained from their sources by any suitable process, for example the isolation processes as described in the above mentioned documents.

10

One possible source of AFP materials is fish. Examples of fish AFP materials are antifreeze glycoproteins (AFGP) (for example obtainable from Atlantic cod, Greenland cod and Tomcod), Type I AFP (for example obtainable from Winter 15 flounder, Yellowtail flounder, Shorthorn sculpin and Grubby sculpin), Type II AFP (for example obtainable from Sea raven, Smelt and Atlantic herring) and Type III AFP (for example obtainable from Ocean Pout, Atlantic wolffish, Radiated shanny, Rock gunnel and Laval's eelpout). A 20 preferred example of the latter type is described in WO 97/02343.

Another possible source of AFP material are invertebrates. Also AFPs may be obtained from Bacteria.

25

A third possible source of AFP material are plants. Examples of plants containing AFPs are garlic-mustard, blue wood aster, spring oat, winter cress, winter canola, Brussels sprout, carrot, Dutchman's breeches, spurge, daylily, winter 30 barley, Virginia waterleaf, narrow-leaved plantain, plantain, speargrass, Kentucky bluegrass, Eastern

- 10 -

cottonwood, white oak, winter rye, bittersweet nightshade, potato, chickweed, dandelion, spring and winter wheat, triticale, periwinkle, violet and grass.

5 Both natural occurring species may be used or species which have been obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFPs and the AFPs may then be used in accordance to the present invention.

10

Genetic manipulation techniques may be used to produce AFPs. Genetic manipulation techniques may be used to produce AFPs having at least 80%, more preferred more than 95%, most preferred 100% homology to the AFPs directly obtained from the natural sources. For the purpose of the invention these AFPs possessing this high level of homology are also embraced within the term "AFPs".

15

The genetic manipulation techniques may be used as follows:
20 An appropriate host cell or organism would be transformed by a gene construct that contains the desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in
25 such a manner that they will be expressed under appropriate conditions (for example in proper orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the
30 art.

- 11 -

A number of expression systems may be utilised to express the polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell culture systems and plants all transformed with the appropriate expression vectors.

A wide variety of plants and plant cell systems can be transformed with the nucleic acid constructs of the desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

For some natural sources the AFPs may consist of a mixture of two or more different AFPs.

Preferably the antifreeze protein is chosen such that it gives an aspect ratio of more than 1.9 to the ice crystal, preferably from 1.9 to 3.0, more preferably from 2.0 to 2.9, even more preferred from 2.1 and 2.8 (see WO 98/04146).

Aspect ratio is defined as the maximum diameter of a particle divided by its minimum diameter. The aspect ratio can be determined by any suitable method. A preferred method is illustrated in the Examples (Example 6).

For the purpose of the invention the preferred AFPs are derived from fish. Especially preferred is the use of fish proteins of the type III, most preferred HPLC 12 as described in our case WO 97/02343.

Ice confections which show the required change in mechanical properties on the addition of the antifreeze protein include

- 12 -

5 unaerated milk containing frozen confections such as ice-cream, frozen yoghurt, and frozen custard, aerated and unaerated sherbet and milk ice, as well as aerated and unaerated frozen confections which do not typically contain milk such as water ices, sorbet, granitas and frozen fruit purees.

Preferably the ice confection is unaerated.

- 10 Preferably the ice confection is selected from a water ice and a milk ice.

15 The ice confection containing the antifreeze protein may constitute the entire product or may be a component of a composite product.

By water ice is meant a frozen solution made essentially from sugar, water, fruit acid or other acidifying agent, colour, fruit or fruit flavouring.

20

By unaerated is meant an ice confection having an overrun of less than 25% (equivalent to 0.2 volume fraction of air), preferably less than 10% (equivalent to 0.09 volume fraction of air). During the processing of the ice confection no deliberate steps such as whipping are undertaken to increase the gas content of the product. However, it should be realised that during normal methods for the preparation of non-aerated ice confections, low levels of gas or air may be incorporated into the product, for example due to the mixing conditions used.

25

30

- 13 -

The ice confection used will typically have an ice content of at least 30% by volume when measured at -18°C , more preferably at least 40% by volume when measured at -18°C , most preferably at least 50% by volume when measured at -18°C .

The ice content may be determined following the techniques described in the article by B de Cindio and S Correra in the Journal of Food Engineering, Volume 24, pages 405-415, 1995.

The enthalpy data required for this technique is obtained using adiabatic calorimetry (Holometrix Adiabatic Calorimeter). The ice contents as expressed herein are measured on an 80g sample poured into the sample holder of the calorimeter and cooled to -75°C by placing the assembly in dry ice prior to placing in the calorimeter (pre-cooled to between -70°C and -80°C). The enthalpy data obtained was analysed to give ice content as a function of the temperature following the method of Cindio and Carrera.

Preferably the ice confection has a total soluble solids content of less than 40% by weight, preferably less than 25% by weight, most preferably less than 15% by weight. For low calorie products the total soluble solids content may be as low as, for example, approximately 5% by weight.

The total soluble solids content is measured at 4°C and is the % by weight of the total composition that is dissolved at that temperature.

- 14 -

It has further been observed that products according to the invention retain their structural integrity for longer when held at ambient temperatures.

5 The ice confectionery product may be a composite product such as a product having 2 or more layers. For example an ice cream core may be coated with one or more layers of water ice. The different layers may be different flavours or colours. A further example would be a sweet composed of two
10 different halves having a different flavour or colour. Each individual component may or may not include AFP. However, it is advantageous for the different components, and in particular components of a different colour or flavour, to include AFP in their composition. It has been observed that
15 products according to the invention which contain AFP demonstrate excellent colour distinction, allowing multi-layered products to be provided which demonstrate no merging of the different colours over time, even when the layers are relatively thin.

20

Description of the Drawings

Figure 1 shows a schematic of the data recorded during a four-point bend test.

25

Figure 2 shows a schematic diagram of typical force vs. displacement curve for a Vickers Hardness Test.

In Figures 3 to 11, AFP containing examples are shown by
30 (□), control samples containing no AFP are shown by (◆)

- 15 -

Figure 3 shows a comparison of the apparent modulus measured for Example 9 compared to Comparative Examples A, B, & C and relevant control samples.

5 Figure 4 shows a comparison of the flexure strength measured for Example 9 compared to Comparative Examples A, B, & C and relevant control samples.

Figure 5 shows a comparison of the Vickers Hardness measured
10 for Example 9 compared to Comparative Examples A, B, & C and relevant control samples.

Figure 6 shows a comparison of the apparent modulus measured for Examples 10-13 compared with a control sample.

15 Figure 7 shows a comparison of the flexure strength measured for Examples 10-13 compared with a control sample.

Figure 8 shows a comparison of the Vickers Hardness measured
20 for Examples 10-13 compared with a control sample.

Figure 9 shows a comparison of the apparent modulus measured for Examples 14-17 compared with a control sample.

25 Figure 10 shows a comparison of the flexure strength measured for Examples 14-17 compared with a control sample.

Figure 11 shows the time taken for consumption of the products of Example 21.

30

- 16 -

Examples

The invention will now be illustrated by means of the following examples.

5

Example 1

Four point bend test

10 The standard four point bend test can be used to determine a number of mechanical properties of ice confection materials. The mechanical properties being measured are Young's modulus (apparent) and flexure strength.

15 In a bend test, a test piece is deformed whilst measuring the applied force and test piece deflection. A schematic data set for an ice confection is shown in Figure 1. The apparent elastic modulus is determined by the gradient of the initial linear part of this curve.

20

The 4-point bend test requires production of a parallel sided rectangular bar of ice confection material. This may be obtained by any suitable means.

25 In this particular application the parallel sided rectangular bar of ice confection was made using aluminium moulds producing bars having the dimensions 25 x 25 x 200 mm.

- 17 -

a) Quiescent frozen ice confections

The liquid ice confection pre-mix was poured into a mould which had been pre-cooled in a blast freezer at -35°C for at least 30 minutes, the mould was then placed in a blast freezer at -35°C for at least 2 hours. The samples were then de-moulded and stored at -25°C until testing (conducted after 5-6 days). 18 to 24 hours prior to testing the samples were equilibrated by storing at -18°C , the temperature at which all tests were performed. A minimum of 10 bars was tested for each sample set and the mean value of each sample set was recorded as the value of the mechanical property being measured.

b) Ice confections frozen with agitation

The ice confection was extruded from the ice cream freezer (scraped surface heat exchanger) at a temperature of from -1°C to -5°C , depending on the ice confection, into a mould which had been pre-cooled in a blast freezer at -35°C for at least 30 minutes, the mould was lined with silicon paper to prevent ice-metal adhesion. The sample was then prepared as above for quiescent frozen samples.

The general test applied to all types of solids is described in "Biomechanics Materials. A practical Approach" Ed. J.F.V. Vincent, Pub. IRL Press, Oxford University Press, Walton Street, Oxford, 1992 and "Handbook of Plastics Test materials" Ed. R.P. Brown, Pub. George Godwin Limited, The Builder Group, 1-3 Pemberton Row, Fleet Street, London, 1981. Testing involves placing each bar onto 2 supports and

- 18 -

bending it until fracture by applying pressure from two upper supports, that are separated by 85mm, centrally on the bar's top surface. The force applied in bending and the displacement of the moving contact are recorded throughout the test. The speed of descent of the moving support was 50mm per minute.

The apparent elastic modulus of the material is given by the equation;

10

$$E = \frac{(0.21) \cdot \text{gradient} \cdot S^3}{BD^3}$$

where the gradient is that shown in Figure 1, S is the span (distance) between the supporting contacts beneath the test bar, B is the width of the bar and D is the depth of the bar.

For these tests the span (S) was 170mm.

20

With reference to Figure 1, the strength of a material under three point bend conditions, is given as;

$$\sigma_u = \frac{(0.75) \cdot F_{\max} S}{BD^2}$$

where σ_u is the flexure strength and F_{\max} is the maximum force recorded.

30

- 19 -

Example 2

Method of determining whether an AFP possesses ice recrystallisation inhibition properties.

5

Recrystallisation inhibition properties can be measured using a modified "splat assay" (Knight et al, 1988). 2.5 µl of the solution under investigation in 30% (w/w) sucrose is transferred onto a clean, appropriately labelled, 16mm circular coverslip. A second coverslip is placed on top of the drop of solution and the sandwich pressed together between finger and thumb. The sandwich is dropped into a bath of hexane held at -80°C in a box of dry ice. When all sandwiches have been prepared, sandwiches are transferred from the -80°C hexane bath to the viewing chamber containing hexane held at -6°C using forceps pre-cooled in the dry ice. Upon transfer to -6°C, sandwiches can be seen to change from a transparent to an opaque appearance. Images are recorded by video camera and grabbed into an image analysis system (LUCIA, Nikon) using a 20x objective. Images of each splat are recorded at time = 0 and again after 60 minutes. The size of the ice-crystals in both assays is compared by placing the slides within a temperature controlled cryostat cabinet (Bright Instrument Co Ltd, Huntington, UK). Images of the samples are transferred to a Quantimet 520 MC image analysis system (Leica, Cambridge UK) by means of a Sony monochrome CCD videocamera. Ice crystal sizing was performed by hand-drawing around ice-crystal. At least 400 crystals were sized for each sample. The ice crystal size was taken as being the longest dimension of the 2D projection of each crystal. The average crystal size was determined as the

10
15
20
25
30

- 20 -

number average of the individual crystal sizes. If the size at 30-60 minutes is similar or only moderately (less than 10%) increased compared to the size at $t=0$, and/or the crystal size is less than 20 micrometer, preferably from 5 to 15 micrometer this is an indication of good ice recrystallisation inhibition properties

Example 3

10 Standard Vickers Hardness test.

The Vickers Hardness test is an indentation test that involves pushing a pyramid shaped indenter into the surface of material and recording the force applied as a function of tip displacement. Force and displacement are measured during the indentation loading cycle and the unloading cycle.

The test is described in "Handbook of Plastics Test materials" Ed. R.P. Brown, Pub. George Godwin Limited, The Builder Group, 1-3 Pemberton Row, Fleet Street, London, 1981.

The Vickers pyramid geometry is an engineering industry standard (BSi 427, 1990). It has an apex angle at the tip of 136°. Hardness is determined as;

$$H_V = \frac{F_{max}}{A}$$

where H_V is the Vickers Hardness, F_{max} is the maximum applied force (see figure 2) and A is the projected area of the

- 21 -

indentation left in the material's surface. The area A is determined by assuming the indentation has the same geometry as the indenter that formed it, i.e. a Vickers pyramid, and therefore the projected area can be determined from the
5 indent depth given by d_i in figure 2.

$$A = 24.5d_i^2$$

The Vickers Hardness of a material is a measure of the
10 material's resistance to plastic deformation.

The test samples were 500ml blocks, manufactured by extruding the ice confection (typically at a temperature of from -1°C to -5°C) from a scraped surface heat exchanger
15 into standard 500ml packets and then placing the packets into a blast freezer at -35°C for two hours prior to storage at -25°C . Prior to testing the samples were equilibrated overnight at the required test temperature of -18°C .

20 Measurements were conducted on a universal testing machine made by Instron (code 4500), within a temperature controlled cabinet at -18°C . The crosshead speed was 2.0mm/min. The maximum load was 95N. The pyramid tip pushed into the surface of the material to a depth of 1.5mm for a water ice
25 and 2.5mm for an ice cream.

- 22 -

Example 4

Measurement of Contiguity

- 5 Contiguity is measured using microstructural images of the ice confection using cryogenic scanning electron microscopy (SEM). Structures are imaged using the technique described in "A low temperature scanning electron microscopy study of ice cream. I. Techniques an general microstructure" Food
10 Structure Vol. 11 (1992), pp 1-9.

In a particulate composite, the Contiguity of the particulate phase is defined as the ratio of the particle to particle interface area divided by the total internal
15 interface area. It is a measure of the degree of network formation of the particulate phase. In ice confections the particles are ice crystals within the matrix and thus contiguity of the ice is defined as;

20
$$C_{ii} = \frac{A_{ii}}{(A_{ii} + A_{im})}$$

where C_{ii} is the contiguity, A_{ii} is the total interfacial surface area of ice-ice interfaces and A_{im} is the interfacial surface area of ice-matrix interfaces. Contiguity can be
25 measured from microstructural images of random planar surfaces cut through the material. Cryo-SEM images of flat fracture surfaces of unaerated ice confection are sufficient for this.

- 23 -

By placing an array of lines on the image of the microstructure, the number of intercepts of these lines with ice-ice and ice-matrix interfaces are counted and combined in the following equation, to give the contiguity;

5

$$C_{ii} = \frac{2N_{ii}}{(2N_{ii} + N_{im})}$$

where N_{ii} = number per unit length of ice-ice intercepts and N_{im} = number per unit length of ice-matrix intercepts.

10

Ideally, approximately 800 interfaces were counted from a total of 5 images that are representative of each sample's structure.

15 To determine contiguity, two sets of measurements were taken from each image. After placing a regular set of lines onto the image, the number of intercepts of the ice-matrix and ice-ice interfaces with these lines were counted, only including all obvious ice-ice interfaces. The count was then
20 repeated, but this time with all possible ice-ice interfaces included. As such, a maximum ice contiguity measurement and a minimum ice contiguity measurement was made for each image. The mean of these figures is then taken as the contiguity value.

25

- 24 -

Example 5

Measurement of Euler-Poincare characteristic

- 5 The Euler-Poincare characteristic is measured using
microstructural images of the ice confection using cryogenic
scanning electron microscopy (SEM). Structures are imaged
using the technique described in " A low temperature
scanning electron microscopy study of ice cream. I.
10 Techniques and general microstructure" Food Structure Vol.
11 (1992), pp 1-9.

- In a two-phase composite structure, the degree of continuity
of one phase can be measured using the Euler-Poincare
15 characteristic. The lower the value of the Euler-Poincare
characteristic for a phase, the more continuous or connected
that phase is within the microstructure. The Euler-Poincare
characteristic can be a positive or negative number. The
definition of the Euler-Poincare characteristic is given in
20 "Unbiased estimation of the Euler-Poincare characteristic"
by B.P. Pinnamaneni, C. Lantuejoul, J.P. Jernot and J.L.
Chermant, Acta Stereologica, 1989, 8/2, p101-106.

- To measure the Euler-Poincare characteristic for ice in ice
25 confections, identification of the ice and matrix phases on
the micro-structural images was performed and using an image
analysis system, the ice phase Euler-Poincare characteristic
was determined using a specifically written analysis
program. Whenever contrast in the images was insufficient
30 for the image analysis system to automatically distinguish
ice and matrix separately, the interface between the two was

- 25 -

identified manually, thus enabling accurate determination of the Euler-Poincare characteristic.

5 The Euler-Poincare characteristic can be measured for ice in an ice confection produced by any processing route.

Example 6

Aspect Ratio Measurement

10

Samples were equilibrated at -18°C in a Prolan environmental cabinet for approximately 12 hours. Microscopic slides were prepared by smearing a thin layer of ice confection from the centre of thin glass plates.

15

Each slide was transferred to a temperature controlled microscopic stage (at -18°C) where images of ice crystals (about 400 individual ice crystals) were collected and relayed through a video camera to an image storage and analysis system.

20

The stored ice crystal images were highlighted manually by drawing around its perimeter which then highlights the whole crystal. Images of the highlighted crystals were then measured using the image analysis software which counts the number of pixels required to complete the longest diameter (length), shortest diameter (breadth), the aspect ratio (length/breadth).

25

30 The average aspect ratio for the crystals was calculated.

- 26 -

Example 7

Production of a "gobstopper" product having different coloured and flavored water ice layers sequentially built up
5 around an ice cream core. The use of water ice composition according to the invention provides a product which is very hard and forces the consumer to lick, rather than bite, the product and so each water ice layer is gradually revealed.

10 Further, the layers of water ice remain discrete and little or no colour "bleed" between layers is observed.

An unaerated sphere of ice cream (20-30mm in diameter) having the following formulation was moulded onto a stick as
15 follows;

Ice cream composition

	% (weight)
20 Double Cream	26.5
Skimmed Milk Powder	9.2
Sucrose	16.0
Water	to 100

25

The ice cream pre-mix was poured into an aluminium mould and the mould was cooled in a blast freezer at -35°C. When the ice cream pre-mix was partially frozen a stick was inserted. When totally frozen the ice cream was demoulded by spraying
30 the outside of the mould with water at 50°C.

- 27 -

The ice cream core was pre-cooled by immersion in solid CO₂ (dry ice) for approximately 2 minutes then dipped into a water ice mix having the following formulation;

5 Water Ice Composition

	% (weight)
Sucrose	15.0
10 Dextrose	5.0
Locust Bean Gum	0.25
Citric Acid	0.5
Flavour / Colour	0.2
Type III AFP*	0.005
15 Water	to 100

* as described in WO 97/02343

Total Solids; 20.5%

Ice Content at -18°C; 68.0% by weight

20

Preparation of the water ice mix

All the water ice ingredients except AFP were mixed together using a high shear mixer for approximately 3 minutes. The
25 water was added at a temperature of 80°C. The temperature of the water ice mix was approximately 55-65°C after mixing.

The AFP was then added, and mixed for approximately 30
seconds in with the hot mix.

30

- 28 -

The mix was then passed through to a plate heat exchanger for pasteurisation at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.

5

The product was then sequentially dipped into water ice mix to build up a number of layers (typically from 12 to 15) of different colours and flavours. Between each dip into the water ice mix the product was cooled in dry ice to facilitate pick-up of the next water ice layer.

10

The resulting product was a sphere of approximately 3 - 5 cm diameter.

15 **Example 8**

Water ice analogues of boiled sweets utilising the hard brittle texture of water ice according to the invention.

20 A water ice mix having the following formulation was prepared as in Example 7;

	Sucrose	15.0
	Dextrose	5.0
25	Locust Bean Gum	0.25
	Citric Acid	0.5
	Flavour / Colour	0.2
	Type III AFP*	0.005
	Water	Balance

30

* as described in WO 97/02343

- 29 -

Total Solids; 20.5% by weight

Ice Content at -18°C; 68.0% by weight

The water ice composition was dosed into rubber or plastic
5 moulds and frozen quiescently in a blast freezer at -35°C.

Example 9, Comparative Examples A to C

An ice cream composition was prepared having the following
10 formulation;

	weight %
Sucrose	13.000
Skimmed Milk Powder	10.000
Butter fat	8.000
15 Maltodextrin 40	4.000
Monoglyceryl Palmitate (MGP)	0.300
Locust Bean Gum	0.144
Carageenan L100	0.016
Flavour	0.012
20 Water	to 100

Total Soluble solids; 35% by weight

Ice content at -18°C; 54% by weight

25 All the ice cream ingredients were mixed together using a
high shear mixer for approximately 3 minutes. The water
being added at a temperature of 80°C. The temperature of the
water ice mix was approximately 55-65°C after mixing.

30 The mix was then homogenized (2000 psi) and passed through
to a plate heat exchanger for pasteurization at 81°C for 25

- 30 -

seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.

After pasteurisation Type III AFP (as described in
5 WO 97/02343) was added to the ice cream pre-mix at a concentration of 0.0025% by weight.

The ice cream pre-mix was then frozen using a Technohoy MF
75 scraped surface heat exchanger such that the following
10 overruns (volume fraction of air (V_{air}) were obtained;

Example 9 - No overrun (0)
Comparative Example A - 43% overrun (0.3)
Comparative Example B - 67% overrun (0.4)
15 Comparative Example C - 100% overrun (0.5)

The ice cream was extruded at a temperature of from -4.4°C to -5.4°C. The product was then hardened in a blast freezer at -35°C, then stored at -25°C.
20

The apparent elastic modulus and the flexure strength were determined using a four point bend test as described in Example 1.

25 Results were compared with relevant control samples containing no AFP. Results are shown in Figures 3 and 4 where AFP containing examples are shown by (\square) and control samples containing no AFP are shown by (\blacklozenge).

30 Δ modulus, Δ modulus/original modulus, Δ strength and

- 31 -

Δ strength/original strength were calculated. Results are shown in Table 1.

The Vickers Hardness was also determined using the method given in Example 3 results are shown in Figure 5 where AFP containing examples are shown by (\square) and control samples containing no AFP are shown by (\blacklozenge).

$\Delta H_v/H_{v \text{ orig}}$ and ΔH_v were calculated. Results are shown in Table 1.

Table 1

Example	ΔE (MPa)	$\Delta E/E_{orig}$	$\Delta \sigma_u$ (MPa)	$\Delta \sigma_u/\sigma_{u \text{ orig}}$	ΔH_v (MPa)	$\Delta H_v/H_{v \text{ orig}}$
9	132	5.1	0.5	2.3	1.25	9.02
A	40	4.7	0.1	1.5	0.2	4.0
B	17	3.3	0.05	1.1	0.15	4.3
C	6.6	1.6	0.02	0.4	0.05	2.1

Examples 10-13

A water ice solution having the following composition was prepared as follows;

	Weight %
Sucrose	20.0
Locust Bean Gum	0.2
Water	to 100

- 32 -

Total soluble solids; 20.2% by weight

Ice content at -18°C; 70% by weight

- 5 All the water ice ingredients were mixed together using a high shear mixer for approximately 3 minutes. The water being added at a temperature of 80°C. The temperature of the water ice mix was approximately 55-65°C after mixing.
- 10 The mix was then passed through to a plate heat exchanger for pasteurization at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.
- 15 After pasteurisation Type III AFP (as described in WO 97/02343) was added to the water ice solution in the following concentrations:

Example 10 - 0.0005%
20 Example 11 - 0.0011%
Example 12 - 0.0025%
Example 13 - 0.005%

- The water ice solution was frozen in a Technohoy MF 75
- 25 scraped surface heat exchanger with no overrun being introduced. The water ice was extruded at a temperature of from -3.9°C to -5.6°C. The product was then hardened in a blast freezer at -35°C, then stored at -25°C.

- 33 -

The apparent elastic modulus and the flexure strength were determined using a four point bend test as described in Example 1.

- 5 Results were compared with a control sample containing no AFP. Results are shown in Figures 6 and 7 where AFP containing examples are shown by (\square) and control samples containing no AFP are shown by (\blacklozenge).
- 10 Δ modulus, Δ modulus/original modulus, Δ strength and Δ strength/original strength were calculated. Results are shown in Table 2.

- The Vickers Hardness was also determined using the method given in Example 3 results are shown in Figure 8 where AFP containing examples are shown by (\square) and control samples containing no AFP are shown by (\blacklozenge).
- 15

- $\Delta H_v/H_{v \text{ orig}}$ and ΔH_v were calculated. Results are shown in Table 2.
- 20

Table 2

Example	ΔE (MPa)	$\Delta E/E_{orig}$	$\Delta \sigma_u$ (MPa)	$\Delta \sigma_u/\sigma_{u \text{ orig}}$	ΔH_v (MPa)	$\Delta H_v/H_{v \text{ orig}}$
10	243	1.14	0.3	0.8	3.8	1.1
11	471	2.2	0.6	1.5	3.2	0.9
12	662	3.1	0.7	1.7	4.0	1.2
13	785	3.7	1.2	3.0	7.0	2.0

- 34 -

Examples 14 - 17

5 A water ice solution having the following composition was prepared;

	Weight %
Sucrose	20.0
Locust Bean Gum	0.2
10 Water	to 100

Total soluble solids; 20.2% by weight

Ice content at -18°C; 70% by weight

- 15 All the water ice ingredients were mixed together using a high shear mixer for approximately 3 minutes. The water being added at a temperature of 80°C. The temperature of the water ice mix was approximately 55-65°C after mixing.
- 20 The mix was then passed through to a plate heat exchanger for pasteurization at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.
- 25 After pasteurisation Type III AFP (as described in WO 97/02343) was added to the water ice solution in the following concentrations;

- 35 -

Example 14 - 0.0005%

Example 15 - 0.0011%

Example 16 - 0.0025%

Example 17 - 0.005%

5

The water ice solution was then quiescently frozen. The water ice solution was poured into the split metal moulds used for the production of mechanical test pieces (see example 1). They were then placed in the cold store overnight to freeze quiescently at a temperature of -25°C. The next day, the test bars were demoulded, placed in polythene bags and stored at -25°C until the day prior to mechanical testing.

15 The apparent elastic modulus and the flexure strength were determined using a four point bend test as described in Example 1.

Results were compared with a control sample containing no AFP. Results are shown in Figures 9 and 10 where AFP containing examples are shown by (□) and control samples containing no AFP are shown by (◆).

Δ modulus, Δ modulus/original modulus, Δ strength and Δ strength/original strength were calculated. Results are shown in Table 3.

- 36 -

Table 3

Example	ΔE (MPa)	$\Delta E /$ E_{orig}	$\Delta \sigma_u$ (MPa)	$\Delta \sigma_u /$ $\sigma_{u orig}$
14	871	2.7	0.85	2.7
15	985	3.1	0.96	3.0
16	1269	4.0	1.4	4.2
17	1347	4.2	1.5	4.8

5 **Examples 18 & 19, Comparative Example D**

A water ice solution having the following composition was prepared as follows;

10	Weight %
	Sucrose 20.0
	Locust Bean Gum 0.2
	Water to 100

- 15 Total soluble solids; 20.2% by weight
Ice content at -18°C ; 70% by weight

All the water ice ingredients were mixed together using a high shear mixer for approximately 3 minutes. The water
20 being added at a temperature of 80°C . The temperature of the water ice mix was approximately $55-65^{\circ}\text{C}$ after mixing.

The mix was then passed through to a plate heat exchanger for pasteurization at 81°C for 25 seconds. The mix was then

- 37 -

cooled to approximately 4°C in the plate heat exchanger prior to use.

After pasteurisation Type III AFP (as described in

5 WO 97/02343) was added to the water ice solution in the following concentrations;

Example 18 - 0.0005% by weight

Example 19 - 0.005 % by weight

10 Comparative Example D - no AFP

The water ice solution was frozen in a Technohoy MF 75 scraped surface heat exchanger with no overrun being introduced. The water ice was extruded at a temperature of
15 from -3.9°C to -5.6°C. The product was then hardened in a blast freezer at -35°C, then stored at -25°C.

Contiguity was measured as in Example 4.

20 Results are shown in Table 4.

Table 4

Example	Contiguity
18	0.24
19	0.22
D	0.11

25

- 38 -

Example 20

A water ice solution having the following composition was prepared as follows;

5

Water Ice Formulation

	% (w/w)
10 Sucrose	10.0
Glucose	5.0
Locust Bean Gum	0.2
Citric Acid	0.5
AFP *	0.005
15 Colour / Flavour	0.2
Water	to 100

* as described in WO 97/02343

- 20 All the water ice ingredients except AFP were mixed together using a high shear mixer for approximately 3 minutes. The water being added at a temperature of 80°C. The temperature of the water ice mix was approximately 55-65°C after mixing.
- 25 The mix was then homogenized (2000 psi) and passed through to a plate heat exchanger for pasteurization at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.
- 30 The mix was then portioned into two halves, and AFP added to one half.

- 39 -

Simple water ice monobites (AFP and non-AFP) were then manufactured as follows;

10ml portions of water ice mix were aliquoted into latex ice
5 cube moulds (hemispherical shape). These were frozen for 2
hours in a blast freezer at -35°C. The moulds were then
transferred to a cold store at -25°C overnight prior to
demoulding. Once demoulded, the monobites were stored in
sealed plastic tubs in a cold store at -25°C. Prior to
10 assessment the water ice monobites were equilibrated to
-18°C overnight. All sensory analysis was carried out at
-18°C.

Sensory Methodology

15 The products were assessed by a highly trained sensory
panel.

A Descriptive Analysis methodology was used whereby trained
panelists identified and quantified the main sensory
20 properties of water ices with and without AFP. The basic
features which this method involves are given in Sensory
Evaluation Techniques, 2nd Edition (1991) M Meilgaard, G. V
Civille and B. T. Carr, CRC Press, and include:

- 25 • The development of the sensory descriptors by the panel,
referred to hereafter as the attribute profile.
- Consensus agreement of common scores on each sensory
attribute of a 'control' product.

- 40 -

- Assessment of some commercial and experimental samples to monitor panel performance prior to the paneling of all the experimental samples.

5 • All tasting sessions are carried out in individual booths, in a controlled environment to eliminate bias from external variables.

10 • Analysis of data via Analysis of Variance (ANOVA) with Duncan's Multiple Range comparison test to check for statistically significant ($p \leq 0.05$) differences between samples.

Sensory Results are shown in Table 5 in which product 1 is the control water ice with no AFP and product 2 is the water
15 ice having 0.005% of Type III AFP

Sensory Mean Scores are listed.

LSD = Least Significant Difference (indicates minimum
20 difference between mean scores for a significant difference for that attribute).

- 41 -

Table 5

Sensory Attribute	Product 1	Product 2	LSD
Hardness	5.63	9.97	0.78
Squeakiness	7.94	5.35	1.05
Crumbiness	5.33	3.51	1.19
Brittleness	5.56	7.96	1.00
Density	3.90	8.02	1.05
Crunchiness	7.72	9.10	0.87
Ice Crystal Detectability	8.07	3.56	1.08
Ice Crystal Size	7.10	2.84	1.06
Roughness	6.50	4.92	0.96
Rate of Melt	6.67	3.96	0.93

5 The water ice with AFP was perceived to be harder, more brittle, denser, more crunchy, less squeaky, less crumbly, with less ice crystals (detection and size), less rough, and have a slower rate of melt in mouth than the water ice with no AFP.

10 Definition of Sensory Attributes

Hardness

The effort required to bite through the sample using the side teeth (1st bite)

15

Squeakiness

The degree to which the sample emits a high pitched squeaking noise when the sample is first bitten

- 42 -

Crumbliness

The degree to which the samples breaks into small, rounded particles when first bitten

5

Brittleness

The degree to which the sample fractures/shears when first bitten

10 Density

The degree to which the sample is perceived to be closely packed, without any air spaces (dense) when first bitten

Crunchiness

- 15 The amount of noise perceived (squeaky, crunchy) during 3 chews with the side teeth

Ice crystal detectability

- 20 The amount of splintery ice crystals detected after 3 chews with the side teeth (assessed by rubbing the sample with the tongue)

Ice crystal size

- 25 The size of ice crystals detected after 3 chews, assessed by rubbing the sample with the tongue

Roughness

The degree to which the sample feels abrasive and coarse after 3 chews with the side teeth

30

- 43 -

Rate of melt

The speed with which the sample can be broken down to a liquid by the side teeth

5 Example 21

Comparison of Time Taken to Consume a Water Ice Product with or without AFP

10 Water Ice Preparation

A water ice solution having the following composition was prepared as follows;

15		% (w/w)
	Sucrose	14.0
	Glucose	6.0
	Stabiliser	0.13
20	Citric Acid	0.3
	AFP *	0.067
	Colour/Flavour	0.3
	Water	to 100

25 * as described in WO 97/02343

All the water ice ingredients except AFP were mixed together using a high shear mixer for approximately 3 minutes. The water being added at a temperature of 80°C. The temperature
30 of the water ice mix was approximately 55-65°C after mixing.

- 44 -

The mix was then homogenized (2000 psi) and passed through to a plate heat exchanger for pasteurisation at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.

5

The mix was then portioned into two halves, and AFP added to one half.

Production of Water Ice Stick Products

10

Simple water ice stick products of approximately 60g were manufactured using conventional water ice moulding technology. Briefly, stainless steel moulds were filled with liquid mix then placed in a glycol bath at approximately -30°C. When the mix had frozen sufficiently sticks were inserted and the mix allowed to freeze completely. Products were demoulded by warming the mould exterior in warm water (ca. 50°C) for approximately 30s and extracting the products from the mould using the stick.

20

Products were then wrapped in waxed paper wrappers and stored at -25°C until use. Prior to consumption, products were equilibrated to -18°C overnight.

25

Consumption of Water Ices

Untrained volunteers were asked to consume either a control or an AFP water ice. Products were selected at random. They were timed whilst doing this and the time taken for the entire product to be consumed was recorded. A week later,

30

- 45 -

the same volunteers were asked to consume whichever product they had not consumed the previous week, and again timed. All products were labeled with batch codes and presented in plain wrappers.

5

The number of volunteers in the study was 41.

Results are shown in Figure 11. The Mean time for Control Water Ice Consumption was 8.4 minutes (Standard Deviation = 0.5). The Mean Time for AFP Water Ice Consumption was 11.8 minutes (Standard Deviation = 0.6).

10

Example 22 Comparative Example E

15 An ice cream composition was prepared having the following formulation;

	Weight %
Sucrose	13.000
Skimmed Milk Powder	10.000
Butter fat	8.000
Maltodextrin 40	4.000
MGP	0.300
Locust Bean Gum	0.144
Carageenan L100	0.016
Flavour	0.012
Water	to 100

Total soluble solids; 35% by weight

20 Ice content at -18°C; 54% by weight

- 46 -

All the ice cream ingredients were mixed together using a high shear mixer for approximately 3 minutes. The water was added at a temperature of 80°C. The temperature of the water ice mix was approximately 55-65°C after mixing.

5

The mix was then homogenized (2000 psi) and passed through to a plate heat exchanger for pasteurisation at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.

10

After pasteurisation Type III AFP (as described in WO 97/02343) was added to the ice cream pre-mix in the following concentrations;

15 Example 22 - 0.005% by weight
Comparative Example E - no AFP

The ice cream pre-mix was then frozen using a Technohoy MF 75 scraped surface heat exchanger, no overrun was introduced
20 into the ice cream. The ice cream was extruded at a temperature of from -4.4 to -5.4°C. The product was then hardened in a blast freezer at -35°C, then stored at -25°C.

25

The Euler-Poincare characteristic was measured in Example 5.

Results are shown in Table 6

- 47 -

Table 6

Example	Euler-Poincare characteristic (mm^{-2})
22	-577
E	339

Example 23, Comparative Example F

5

A water ice solution having the following composition was prepared as follows;

	Weight %
Sucrose	20.0
Locust Bean Gum	0.2
Water	to 100

- 10 Total soluble solids; 20.2% by weight
Ice content at -18°C ; 70% by weight

The water ice solution was prepared as in Example 13.

- 15 After pasteurisation Type III AFP (as described in WO 97/02343) was added to the water ice solution in the following concentrations;

Example 23 - 0.005% by weight

- 20 Comparative Example F - no AFP

The water ice solution was frozen quiescently as described in Example 17 with no overrun being introduced.

- 48 -

The Euler-Poincare characteristic was measured as in Example 5.

Results are shown in Table 7

5

Table 7

Example	Euler-Poincare characteristic (mm^{-2})
23	-383
F	4.3

10 **Example 24**

A milk ice solution having the following composition was prepared as follows;

15

	% (w/w)
Sucrose	13.2
Corn Syrup	2.8
Whole Milk Powder	11.2
Skimmed Milk Powder	5.0
Stabiliser	0.27
Emulsifier	0.20
Colour/Flavour	0.06
AFP*	0.005
Water	to 100

* as described in WO 97/02343

- 49 -

All the milk ice ingredients except AFP were mixed together using a high shear mixer for approximately 3 minutes. The water was added at a temperature of 80°C. The temperature of the milk ice mix was approximately 55-65°C after mixing.

5

The mix was then homogenized (2000 psi) passed through to a plate heat exchanger for pasteurisation at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.

10

The mix was then portioned into two halves, and Type III AFP (as described in WO 97/02343) added to one half.

15

The milk ice solutions were then quiescently frozen. The milk ice solution was poured into the split metal moulds used for the production of mechanical test pieces (see example 1). They were then placed in the cold store overnight to freeze quiescently at a temperature of -25°C. The next day, the test bars were demoulded, placed in polythene bags and stored at -25°C until the day prior to mechanical testing.

20

The apparent elastic modulus and the flexure strength were determined using a four point bend test as described in Example 1.

25

Results for the milk ice containing AFP were compared with the control sample containing no AFP.

30

Δ modulus, Δ modulus/original modulus, Δ strength and

- 50 -

Δ strength/original strength were calculated. Results are shown in Table 8.

Table 8

5

Example	ΔE (MPa)	$\Delta E /$ E_{orig}	$\Delta \sigma_u$ (MPa)	$\Delta \sigma_u /$ $\sigma_{u orig}$
24	756	16.2	1.81	6.68

Example 25

10 A milk ice solution having the following composition was prepared as follows;

	% Weight
Sucrose	13.2
Corn Syrup	2.8
Whole Milk Powder	11.2
Skimmed Milk Powder	5.0
Stabiliser	0.27
Emulsifier	0.20
Colour/Flavour	0.06
AFP*	0.005
Water	to 100

*as described in WO 97/02343

15 All the milk ice ingredients except AFP were mixed together using a high shear mixer for approximately 3 minutes. The

- 51 -

water being added at a temperature of 80°C. The temperature of the milk ice mix was approximately 55-65°C after mixing.

5 The mix was then homogenized (2000 psi) and passed through to a plate heat exchanger for pasteurisation at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.

10 The mix was then portioned into two halves, and Type III AFP (as described in WO 97/02343) added to one half.

15 The milk ice solutions were frozen in a Technohoy MF 75 scraped surface heat exchanger with no overrun being introduced. The water ice was extruded at a temperature of from -3.9°C to -5.6°C. The product was then hardened in a blast freezer at -35°C, then stored at -25°C.

The apparent elastic modulus and the flexure strength were determined using a four point bend test as described in Example 1.

20

Results for the milk ice containing AFP were compared with the control sample containing no AFP.

25 Δ modulus, Δ modulus/original modulus, Δ strength and Δ strength/original strength were calculated. Results are shown in Table 9.

- 52 -

Table 9

Example	ΔE (MPa)	$\Delta E /$ E_{orig}	$\Delta \sigma_u$ (MPa)	$\Delta \sigma_u /$ $\sigma_{u orig}$
25	290	5.08	0.968	4.17

5 Example 26

An ice cream composition was prepared having the following formulation;

	weight %
10 Sucrose	13.500
Skimmed Milk Concentrate (30% solids)	24.000
Cream (40% fat)	43.000
Egg Yolk (unsweetened)	4.500
Vanilla flavour	1.000
15 Water	to 100

Total soluble solids; 45% by weight

Ice content at -18°C ; 46% by weight

- 20 All the ice cream ingredients were mixed together using a high shear mixer for approximately 3 minutes. The water being added at a temperature of 80°C . The temperature of the ice cream mix was approximately $38-45^{\circ}\text{C}$ after mixing.
- 25 The mix was then homogenized (2000 psi) and passed through to a plate heat exchanger for pasteurization at 81°C for 25

- 53 -

seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.

After pasteurisation Type I AFP (from AF Protein) was added
5 to the ice cream pre-mix in the following concentration;

Example 26 - 0.001% by weight

The ice cream pre-mix was then frozen using a Technohoy MF
10 75 scraped surface heat exchanger with no overrun being introduced. The ice cream was extruded at a temperature of from -4.8°C to -5.5°C. The product was then hardened in a blast freezer at -35°C, then stored at -25°C.

15 The apparent elastic modulus and the flexure strength were determined using a four point bend test as described in Example 1.

Results were compared with relevant control sample
20 containing no AFP.

Δ modulus, Δ modulus/original modulus, Δ strength and Δ strength/original strength were calculated. Results are shown in Table 10.

25

Table 10

Example	ΔE (MPa)	$\Delta E /$ E_{orig}	$\Delta \sigma_u$ (MPa)	$\Delta \sigma_u /$ $\sigma_{u orig}$
26	138	4.2	0.408	1.09

5 **Example 27**

A water ice solution having the following composition was prepared as follows;

	% by weight
10 Sucrose	18
Locust Bean Gum	0.18
Hydrolysed milk protein (Hyfoama DS**)	0.1
Grass AFP solution*	30.0
15 water	to 100

* as described in WO 98/04699. The amount of grass AFP solution used was determined by the minimum amount required to provide the recrystallisation inhibition as defined by

20 Example 2.

** Hyfoama DS is a trademark of Quest International

Total soluble solids; 18% by weight

25 Ice Content at -18°C; 73% by weight

- 55 -

A control mix was made without AFP, the grass AFP solution being replaced by 30% by weight water.

5 All the water ice ingredients except AFP were mixed together using a high shear mixer for approximately 3 minutes. The water being added at a temperature of 80°C. The temperature of the water ice mix was approximately 55-65°C after mixing.

10 The mix was then passed through to a plate heat exchanger for pasteurization at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.

15 The AFP was added after pasteurisation.

The water ice solution was frozen in a Technohoy MF 75 scraped surface heat exchanger with no overrun introduced. The water ice was extruded at a temperature of from -2.6°C to -3.6°C. The product was then hardened in a blast freezer
20 at -35°C, then stored at -25°C.

The apparent elastic modulus and the flexure strength were determined using a four point bend test as described in Example 1.

25 Results were compared with the control sample containing no AFP.

30 Δ modulus, Δ modulus/original modulus, Δ strength and Δ strength/original strength were calculated. Results are shown in Table 11.

Table 11

Example	ΔE (MPa)	$\Delta E /$ E_{orig}	$\Delta \sigma_u$ (MPa)	$\Delta \sigma_u /$ $\sigma_{u orig}$
27	230	0.84	0.615	1.03

5

Example 28

A water ice solution having the following composition was
10 prepared as in Example 14;

A water ice solution having the following composition was
prepared as follows;

15		% by weight
	Sucrose	18
	Locust Bean Gum	0.18
	Hydrolysed milk protein (Hyfoama DS**)	0.1
	Grass AFP solution*	30.0
20	water	to 100

* as described in WO 98/04699. The amount of grass AFP
solution used was determined by the minimum amount required
to provide the recrystallisation inhibition as defined by
25 Example 2.

** Hyfoama DS is a trademark of Quest International
Total soluble solids; 18% by weight
Ice Content at -18°C; 73% by weight

- 57 -

A control mix was made without AFP, the grass AFP solution being replaced by 30% by weight water.

- 5 All the water ice ingredients except AFP were mixed together using a high shear mixer for approximately 3 minutes. The water being added at a temperature of 80°C. The temperature of the water ice mix was approximately 55-65°C after mixing.
- 10 The mix was then passed through to a plate heat exchanger for pasteurization at 81°C for 25 seconds. The mix was then cooled to approximately 4°C in the plate heat exchanger prior to use.
- 15 After pasteurisation the AFP solution was added to the water ice solution.

- The water ice solution was then quiescently frozen. The water ice solution was poured into the split metal moulds
- 20 used for the production of mechanical test pieces (see example 1). They were then placed in the cold store overnight to freeze quiescently at a temperature of -25°C. The next day, the test bars were demoulded, placed in polythene bags and stored at -25°C until the day prior to
 - 25 mechanical testing.

The apparent elastic modulus was determined using a four point bend test as described in Example 1.

- 30 Results were compared with the control sample containing no AFP.

- 58 -

Δ modulus and Δ modulus/original modulus were calculated.
Results are shown in Table 12.

5 **Table 12**

Example	ΔE (MPa)	$\Delta E /$ E_{orig}
28	146	2.59

- 59 -

Claims

1. An ice confection product having a volume of from 1 to 20 ml and a thickness of greater than 5mm comprising an antifreeze protein, wherein
5 Δ modulus/original modulus ≥ 0.4 , and/or
 Δ strength/original strength ≥ 0.4 ; providing that when Δ modulus/original modulus ≤ 6.0 , Δ modulus ≥ 50 MPa, and/or when Δ strength/original strength ≤ 2.0 ,
10 Δ strength ≥ 0.2 MPa.
2. An ice confection according to claim 1 wherein
 Δ modulus/original modulus ≥ 0.4 ; providing that when Δ modulus/original modulus ≤ 6.0 , Δ modulus ≥ 90 MPa.
15
3. An ice confection according to claim 1 wherein
 Δ modulus/original modulus ≥ 1.0 ; providing that when Δ modulus/original modulus ≤ 6.0 , Δ modulus ≥ 100 MPa.
- 20 4. An ice confection according to claim 1 wherein
 Δ strength/original strength ≥ 0.7 , preferably ≥ 1.5 .
5. An ice confection according to any preceding claim, wherein the antifreeze protein is present at a concentration
25 of from 0.0005% by weight to 0.005% by weight.
6. An ice confection according to any preceding claim, wherein the ice confection is unaerated.

- 60 -

7. An ice confection according to any preceding claim,
wherein the ice confection is water ice or milk ice.
8. An ice confection product according to any preceding
5 claim, wherein the antifreeze protein is chosen such that it
gives an aspect ratio of more than 1.9 to the ice crystal.
9. An ice confection product according to any preceding
claim, wherein the antifreeze protein is Type III AFP as
10 described in WO 97/02343.
10. An ice confection product according to any preceding
claim wherein the ice confection has an ice content of at
least 30% by volume, preferably at least 40% by volume, most
15 preferably at least 50% by volume, when measured at -18°C .
11. An ice confection product according to any preceding
claim wherein the ice confection has a total soluble solids
level of less than 40% by weight, preferably less than 25%
20 by weight, most preferably 15% by weight.
12. An ice confection product according to any preceding
claim having a contiguity of at least 0.2 and an ice content
of from 50 to 90% by weight when measured at -18°C .
25
13. An ice confection product according to any preceding
claim having a Euler-Poincare characteristic of less than
 -150mm^{-2} and an ice content of from 50 to 90% by weight when
measured at -18°C .
30

- 61 -

14. Use of an ice confection according to any preceding claim to provide an ice confection product which is long-lasting in the mouth.

1/5

Fig.1.

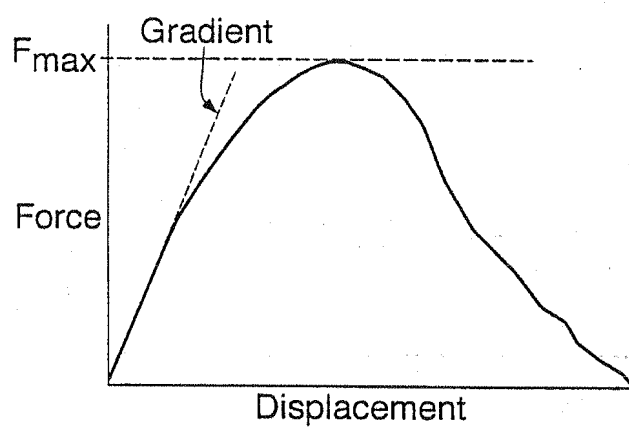
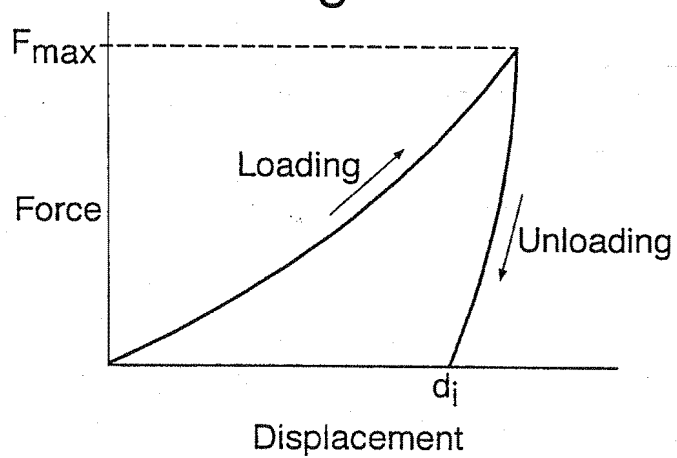


Fig.2.



2/5

Fig.3.

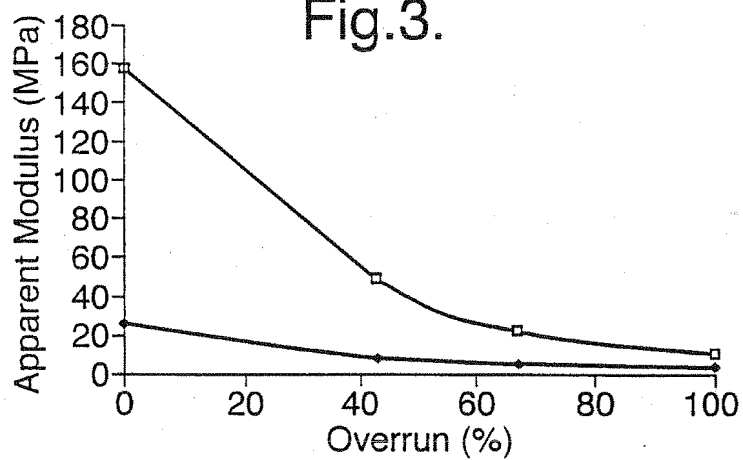


Fig.4.

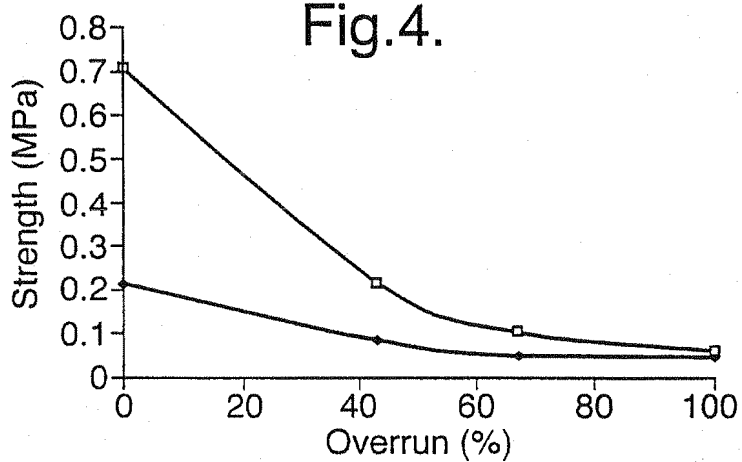
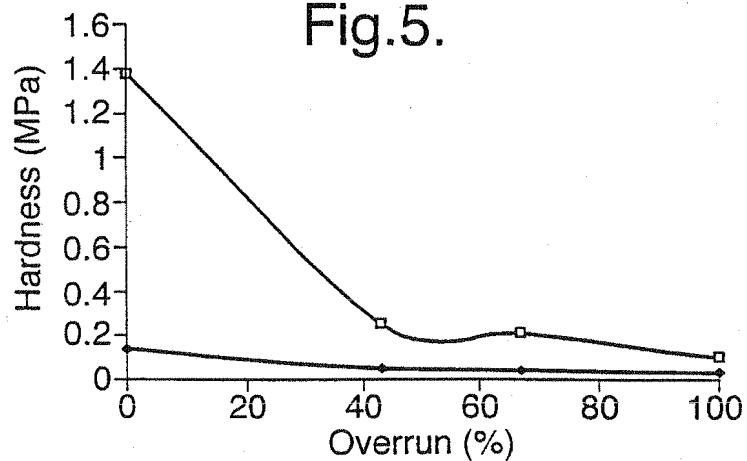
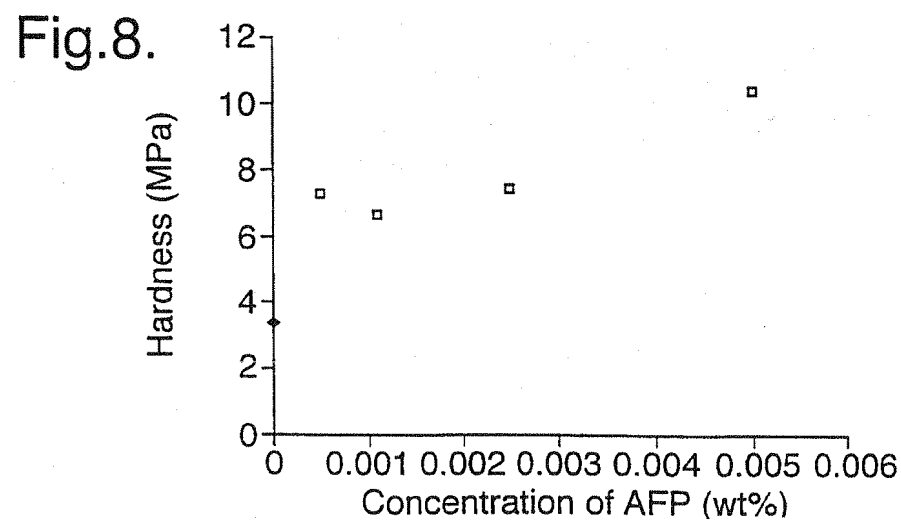
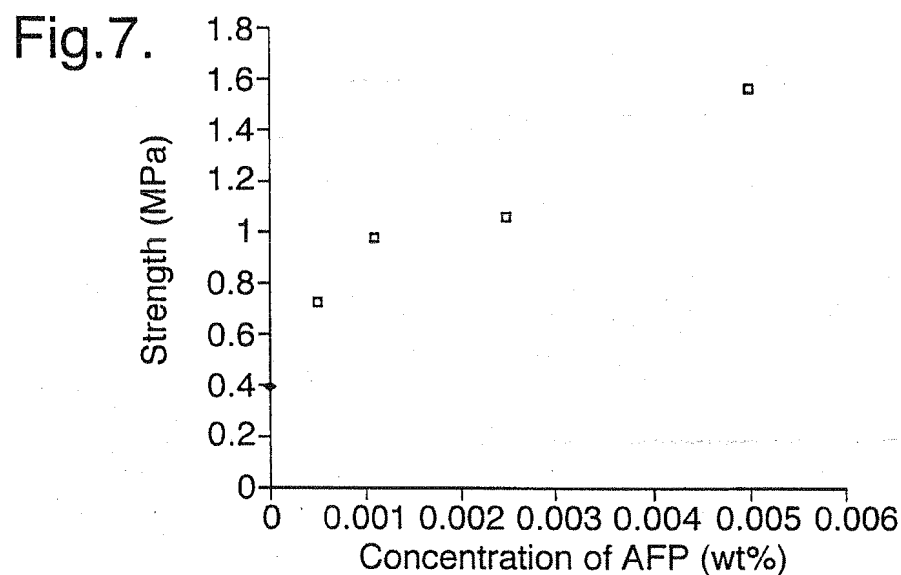
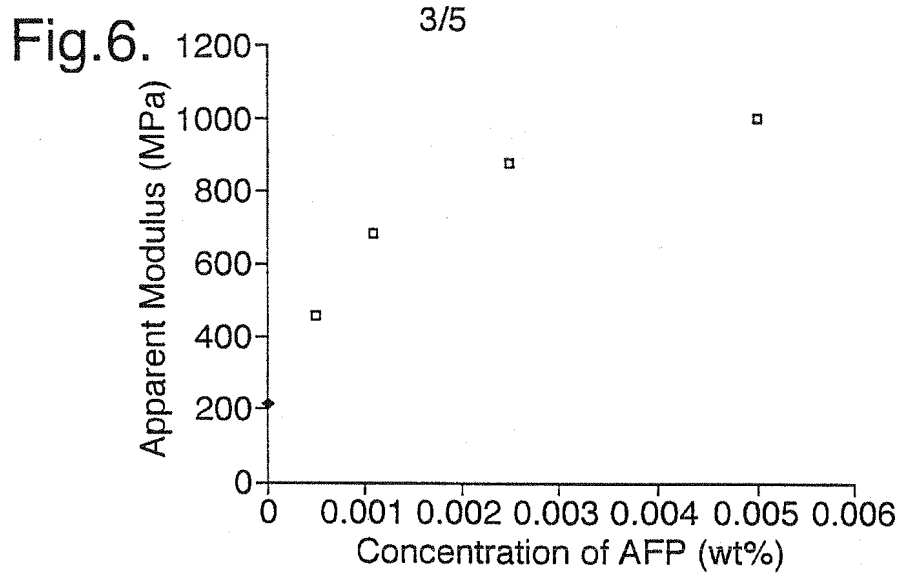


Fig.5.





4/5

Fig.9.

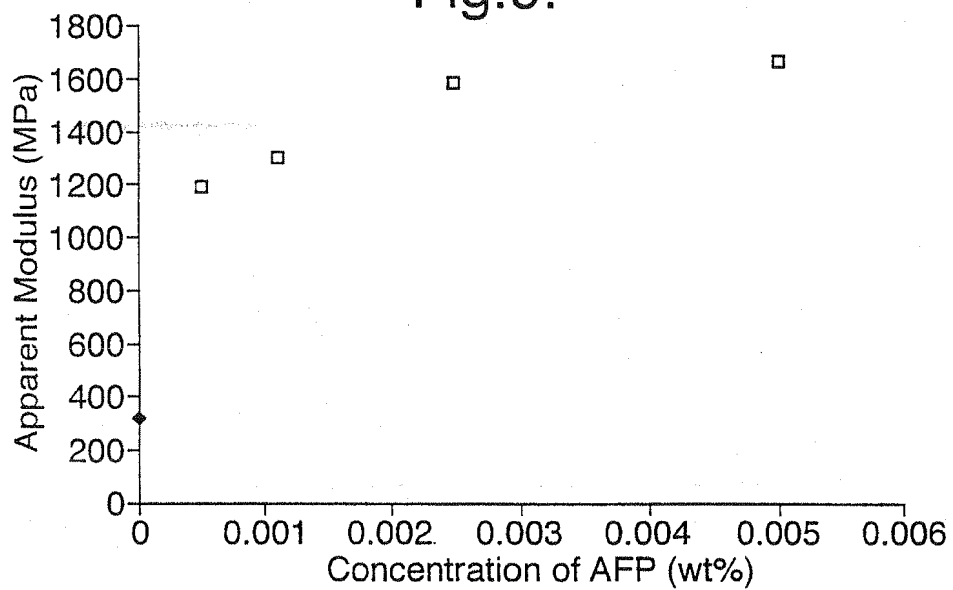
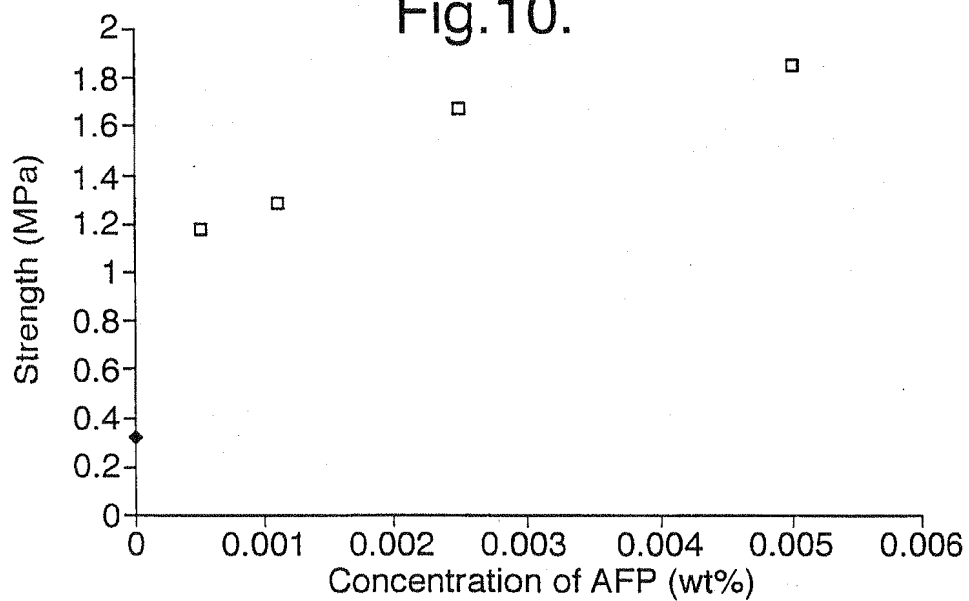
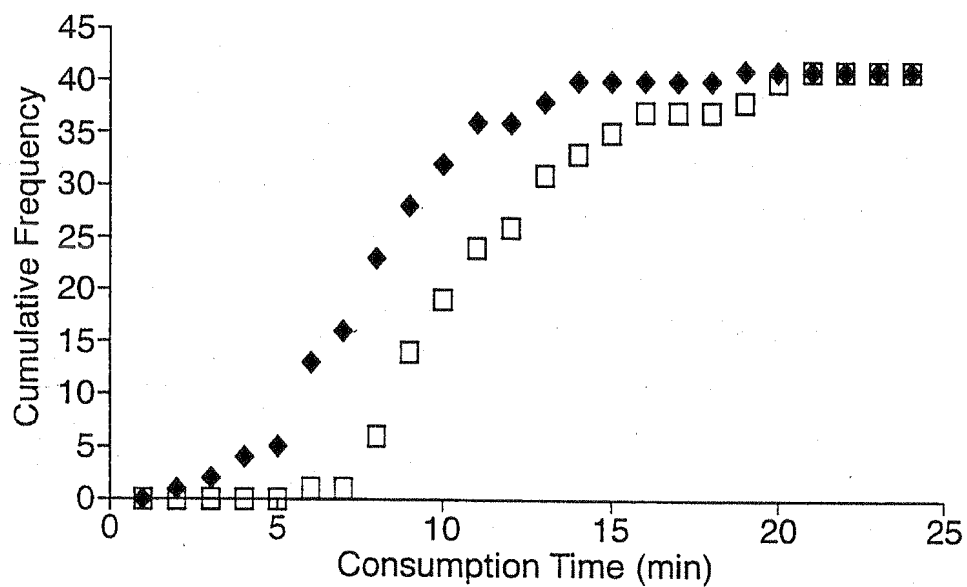


Fig.10.



5/5

Fig.11.



INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/01760

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23G9/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 02343 A (UNILEVER PLC ;UNILEVER NV (NL)) 23 January 1997 (1997-01-23)	1,5-14
Y	claims 1,6,24	2-4
Y	DE 197 32 132 A (UNILEVER NV) 29 January 1998 (1998-01-29) page 7, line 1 - line 25; example 6	2-4
X	WO 98 41109 A (UNILEVER PLC ;UNILEVER NV (NL)) 24 September 1998 (1998-09-24) claims; examples	1
X	WO 98 41106 A (UNILEVER PLC ;UNILEVER NV (NL)) 24 September 1998 (1998-09-24) examples	1
	--- -/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

17 May 2000

Date of mailing of the international search report

29.05.2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Guyon, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01760

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 620 732 A (CLEMMINGS JOHN F ET AL) 15 April 1997 (1997-04-15) claims; examples ---	1
A	DE 197 32 135 A (UNILEVER NV) 26 February 1998 (1998-02-26) examples ---	
A	WO 92 22581 A (UNIV WATERLOO) 23 December 1992 (1992-12-23) -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/01760

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-14
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-14

Present claims 1-14 relate to a product and method by reference to the following parameters: dimensions, Young's modulus, strenght, crystal aspest ratio, contiguity, Euler-Poincare characteristic and long lasting character.

The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible.

Consequently, the search has been restricted to: Use of antifreeze protein (e.g. AFP type III HPLC-12) in ice-creams corresponding as closely as possible to the formulation of examples 7-28.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/01760

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9702343 A	23-01-1997	AU 6519096 A	05-02-1997
		BR 9609325 A	25-05-1999
		CA 2226101 A	23-01-1997
		CN 1193999 A	23-09-1998
		CZ 9800006 A	13-05-1998
		EP 0836646 A	22-04-1998
		HU 9802321 A	01-02-1999
		JP 11508451 T	27-07-1999
		PL 324437 A	25-05-1998
		SK 179497 A	08-07-1998
DE 19732132 A	29-01-1998	AU 3621297 A	20-02-1998
		AU 3621397 A	20-02-1998
		CN 1226138 A	18-08-1999
		CN 1231580 A	13-10-1999
		CZ 9900250 A	14-07-1999
		CZ 9900254 A	14-07-1999
		DE 19732136 A	29-01-1998
		WO 9804147 A	05-02-1998
		WO 9804148 A	05-02-1998
		EP 0923306 A	23-06-1999
		EP 0959689 A	01-12-1999
		FR 2751513 A	30-01-1998
		FR 2751514 A	30-01-1998
		GB 2315753 A	11-02-1998
		GB 2315662 A	11-02-1998
		IT MI971754 A	25-01-1999
		IT MI971755 A	25-01-1999
		PL 331376 A	05-07-1999
		PL 331428 A	19-07-1999
		SK 8899 A	11-06-1999
		SK 9099 A	11-06-1999
		ZA 9706472 A	22-01-1999
WO 9841109 A	24-09-1998	AU 3621397 A	20-02-1998
		AU 7037098 A	12-10-1998
		AU 7207998 A	12-10-1998
		CN 1231580 A	13-10-1999
		CZ 9900254 A	14-07-1999
		DE 19732136 A	29-01-1998
		WO 9804148 A	05-02-1998
		WO 9841107 A	24-09-1998
		EP 0959689 A	01-12-1999
		FR 2751513 A	30-01-1998
		GB 2315753 A	11-02-1998
		IT MI971755 A	25-01-1999
		PL 331428 A	19-07-1999
		SK 9099 A	11-06-1999
WO 9841106 A	24-09-1998	AU 6831698 A	12-10-1998
		EP 0966206 A	29-12-1999
		AU 3621397 A	20-02-1998
		CN 1231580 A	13-10-1999
		CZ 9900254 A	14-07-1999
		DE 19732136 A	29-01-1998
		WO 9804148 A	05-02-1998
		EP 0959689 A	01-12-1999
		FR 2751513 A	30-01-1998

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/01760

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9841106 A		GB 2315753 A IT MI971755 A PL 331428 A SK 9099 A	11-02-1998 25-01-1999 19-07-1999 11-06-1999
US 5620732 A	15-04-1997	AU 704570 B AU 5790496 A CA 2195950 A EP 0783254 A JP 10508759 T WO 9639878 A US 6017574 A	29-04-1999 30-12-1996 19-12-1996 16-07-1997 02-09-1998 19-12-1996 25-01-2000
DE 19732135 A	26-02-1998	AU 3443797 A AU 3621297 A AU 3621397 A AU 3693497 A CN 1226284 A CN 1226138 A CN 1226139 A CN 1231580 A CZ 9900250 A CZ 9900251 A CZ 9900252 A CZ 9900254 A DE 19732136 A WO 9804699 A WO 9804146 A WO 9804147 A WO 9804148 A EP 0918863 A EP 0923306 A EP 0959689 A EP 0924990 A FR 2751657 A FR 2751513 A GB 2315752 A GB 2315753 A IT MI971752 A IT MI971755 A PL 331376 A PL 331377 A PL 331428 A PL 331483 A SK 8899 A SK 8999 A SK 9099 A SK 9499 A ZA 9706473 A ZA 9706477 A	20-02-1998 20-02-1998 20-02-1998 20-02-1998 18-08-1999 18-08-1999 18-08-1999 13-10-1999 14-07-1999 14-07-1999 14-07-1999 14-07-1999 29-01-1998 05-02-1998 05-02-1998 05-02-1998 05-02-1998 02-06-1999 23-06-1999 01-12-1999 30-06-1999 30-01-1998 30-01-1998 11-02-1998 11-02-1998 25-01-1999 25-01-1999 05-07-1999 05-07-1999 19-07-1999 19-07-1999 11-06-1999 12-07-1999 11-06-1999 11-06-1999 22-01-1999 25-01-1999
WO 9222581 A	23-12-1992	AU 1907192 A CA 2110510 A EP 0589928 A US 5852172 A US 5972679 A	12-01-1993 23-12-1992 06-04-1994 22-12-1998 26-10-1999